LITERATURE REVIEW
Catharanthus roseus (L.) G. Don.

History and geographical distribution

*Catharanthus roseus* (L.) G. Don., known in trade as Vinca, shot into prominence as a medicinal plant due to accidental discovery of antineoplastic activities of leaf alkaloids by Nobel *et al.* (1958) and its elevation from the position of an adulterant in *Rauvolfia* roots to an alternate source for raubasin and serpentine alkaloids used in the preparation of hypertension-relieving drugs. *C. roseus* also enjoys the distinction of a taxon possessing largest number of alkaloids in the plant kingdom (Hui-Lin Li and Willaman, 1972). The popularity of *C. roseus* as an ornamental plant has earned its vernacular names, such as Sadabahar, Sadhaphul, Nayantara, Rattan jot, Billaganneru, Gul Feringhi, Sudukadu mallikai, Nityakalyani, Ainskati, etc. (Stearn, 1966, 1975; Krishnan, 1995). The plant is indigenous to Madagascar and has been naturalised in all tropical and subtropical areas of the world. The plant is under cultivation in Madagascar, India, Israel and USA (Anonymous, 1982; Stearn, 1975). In India, it is found growing wild along the sandy coastal tracts of Tamil Nadu and Kerala States (Gupta, 1977).

Botany

The plant was previously called *Vinca rosea* Linn. and *Lochnera rosea* (L.) Reichb., as well. However, after studying the floral characters of *Vinca*
and *Catharanthus*, the plant was renamed as *Catharanthus roseus* G. Don. The plant belongs to family Apocyanaceae. It is an erect, evergreen, everblooming herb or sub-shrub, 40-80 cm high and wide at the base. The branching starts from the base (Fig. 1a and 1b). The plant is pubescent when young and glabrous when mature. The leaves are petiolate, opposite or alternate, thick and leathery, 3-8 cm long and 1.5 - 5.0 cm broad, oblong or obovate, base acute while apex is rounded to mucronate and margin entire. Veins are prominent on the lower surface. The colour of the upper surface of the leaves is deep green, while that of lower surface is much lighter. The petiole has a pinkish tinge. Flowers are borne on axils in pairs; violet or rose (var. *roseus*), white (var. *albus*) or white with red eye (var. *ocellatus*). The calyx lobes are linear-subulate. The corolla tube is cylindrical (Flory, 1944; Farnsworth, 1962; Kulkarni *et al.*, 1984). The epipetalous anthers are borne on short filaments inside the bulging distal end of corolla tube converging conically above the stigma. The bicarpellate ovary is basally distinct with fused common style and stigma, which is ascribed to post-genital carpel fusion (Walker, 1975). The dehiscent fruit consist of a pair of follicles, each measuring 25 mm in length and 2.3 mm in diameter, containing up to 30 linearly arranged seeds with a thin black tegumen. On maturity, the follicles split along the length dehiscing the seeds (Krishnan, 1995).

**Cytogenetics**

The haploid chromosome number was determined by Furusto as n = 8 (cf. Darlington and Janaki Ammal, 1945). The somatic karyotype was described by Dhyansagar and Sudhakaran (1966). They also studied meiosis in pollen mother cells of both pink and white varieties, and found similarity.
Fig. 1a,b. *Catharanthus roseus*: (a) a flowering plant (var. *albus*); (b) a field view.
in chiasma frequency. It is a naturally self-pollinated species. However, initial crossing studies have shown that although the plant is self pollinated, frequent out crossing has been observed resulting into different intermediate types (Gjerstad, 1965; Krishnan et al., 1979). Artificial tetraploids have been produced by a number of workers (Cross and Johnson, 1947; Dhyansagar and Sudhakaran, 1970; Gogitize and Daptev, 1981; Kulkarni et al., 1984). Kulkarni (1984) has reported the increased resistance in tetraploids of *Catharanthus roseus* towards die-back disease.

**Crop cultivation**

The plant can grow in a variety of environmental conditions. However, it grows well under tropical and subtropical climatic conditions. Light sandy loam soil is preferred for its commercial cultivation, although it can grow on all types of soil except waterlogged and highly alkaline soils where the growth is retarded (Virmani et al., 1978). A well distributed rainfall of about 100 cm is very beneficial for rainfed crop (Datta and Patani, 1976; Virmani et al., 1978). The plant can be propagated vegetatively or by seeds. However, it is usually propagated through seeds. Seeds can be directly sown in the field, but raising a nursery and transplanting the seedlings in the properly prepared field is better (Virmani et al., 1978). Best season for planting is the onset of monsoon (Bhattacharjee, 2000).

**Chemistry, uses and biological activity**

More than 100 alkaloids have been isolated from leaves and roots of periwinkle. Alkaloids are extracted from various plant parts using solvent extraction procedure. Different methodologies have been adapted for the
extraction of alkaloids namely, solvent extraction, selective or differential extraction, gradient pH extraction and superficial carbon dioxide extraction (Virmani et al., 1978; Lee et al., 1992; Shukla et al., 1997).

Based on traditional knowledge of folklore of different localities, various medicinal uses of the plant viz., anti-diabetic, abortive, purgative, diaphoretic, hemostatic, anti-malarial, menstrual regulating, vermifuge, wound healing, anti-leukemic and cure of skin infection, dysentery, dyspepsia and toothache, have been reported (Guerrero, 1921; Aldaba and Oliveros-Belardo, 1938; Chopra, 1949; Neogi and Bhatia, 1956; Virmani et al., 1978). Plant extracts and alkaloids have also been reported to possess numerous bioactive properties (Table-1).

Table-1: Biological activity of alkaloids and plant extracts of Catharanthus roseus

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Activity</th>
<th>Material/alkaloid component(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Anti-bacterial</td>
<td>Crude alkaloid</td>
<td>Chopra et al. (1949)</td>
</tr>
<tr>
<td>3.</td>
<td>Anti-diuretic</td>
<td>Plant extract</td>
<td>Neogi and Bhatia (1956)</td>
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<tr>
<td>4.</td>
<td>Anti-diabetic</td>
<td>Plant extract</td>
<td>Chopra (1949), Neogi and Bhatia (1956)</td>
</tr>
<tr>
<td>7.</td>
<td>Anti-helminthic</td>
<td>Plant extract</td>
<td>Neogi and Bhatia (1956)</td>
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<th>S. No.</th>
<th>Activity</th>
<th>Material/alkaloid component(s)</th>
<th>Reference(s)</th>
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<tr>
<td>8.</td>
<td>Anti-microbial</td>
<td>Strictosidine</td>
<td>Luijendijk et al. (1996)</td>
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<td></td>
<td>and anti-feedant</td>
<td></td>
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</tr>
<tr>
<td>9.</td>
<td>Anti-mitotic</td>
<td>Leaf extract, Vinflunine</td>
<td>Merzabani et al. (1979),</td>
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<td></td>
<td></td>
<td></td>
<td>Kruczynski et al. (1998)</td>
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<tr>
<td>10.</td>
<td>Anti-viral</td>
<td>Crude alkaloid</td>
<td>Farnsworth et al. (1968)</td>
</tr>
<tr>
<td>11.</td>
<td>Hypolipidaemic</td>
<td>Leaf extract</td>
<td>Kulkarni et al. (1994),</td>
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<td></td>
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<td></td>
<td>Mukherjee et al. (1995)</td>
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<td>Chandravadana et al. (1994)</td>
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**Occurrence of fungal, bacterial and phytoplasma diseases on Catharanthus roseus**

**Fungal Diseases**

**i. Collar /Root rot**

Ravindra (1986) observed a high incidence of collar and root rot disease on Catharanthus roseus at a Research Station of the Central Institute of Medicinal and Aromatic Plants (CIMAP) at Bangalore and its adjoining areas during the rainy season. The isolations made from the collar and root regions of infected plants always yielded *Pythium aphanidermatum*. He established pathogenicity of *P. aphanidermatum* on the healthy plants by artificial inoculation. WenChuan et al. (1998) also noted the occurrence of *Fusarium* root rot of periwinkle in Taiwan. Diseased plants showed stunting, yellowing of lower leaves and reddish brown discoloration on the tap roots.
Discoloration of the vascular bundles was observed at the basal portion of
the infected stem. They also described the pathogen morphology, cultural
characteristics and effect of temperature on mycelial growth. Mc Govern and
Seijo (1999) observed a severe black root rot in plotted Catharanthus roseus.
The causal organism was identified as Thielaviopsis basicola and its
pathogenicity was confirmed.

ii. Die-back

Janardhanan et al. (1977) reported the occurrence of die-back disease
caused by Pythium butleri in the commercial plantations of C. roseus during
rainy season. They have also described symptomatology and etiology of the
disease.

iii. Flower spot

Holcomb (1998) observed dark gray flower spots on 18 cultivars of
periwinkle that often coalesced and led to blighting of flowers under high
humid conditions. Fungi were isolated from flower spots by placing necrotic
tissue on potato-dextrose agar (PDA). A fungus that produced cottony, white
mycelium and black spore masses was consistently isolated from diseased
tissue and identified as Choanephora cucurbitarum. Pathogenicity was
confirmed by inoculation of detached flowers and flowers on intact
periwinkle plants.

iv. Leaf spot

Goyal and Pathak (1982) reported that in monsoon season,
plantations of Catharanthus roseus were severely damaged by a leaf spot
disease caused by *Alternaria alternata*. The symptoms appeared in the form of small light brown spots over entire leaf surface. In advanced stage of infection, the spots turned into necrotic lesions with concentric rings.

v. **Seedling blight/Leaf and Stem blight/Stem rot/Grey mould**

CheinWei *et al.* (1997) reported the occurrence of five fungal diseases from Taiwan. These were recognised as seedling blight (*Rhizoctonia solani*) resembling with type specimen of *R. solani* AG-4, leaf and stem blight/rot (*Phytophthora parasitica* = *P. nicotianae* var. *parasitica*), stem rot (*Sclerotium rolfsii* = *Corticium rolfsii*), grey mould (*Botrytis cineria*) and Sclerotinia rot (*Sclerotinia sclerotiorum*). Grey mould and Sclerotinia rot appeared during the winter, while seedling blight, leaf/stem blight and stem rot occurred in the summer. Ou-Yang (1998) identified 9 fungi namely, *Botrytis cineria*, *Colletotrichum gloesporioides* (*Glomerella cingulata*), *Fusarium oxysporum*, *F. solani*, *Phytophthora parasitica*, (*P. nicotianae* var. *parasitica*), *Pythium aphanidermatum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* (*Corticium rolfsii*), pathogenic to *Catharanthus roseus* seedlings with *P. parasitica* (*P. nicotianae* var. *parasitica*) as the most damaging one.

vi. **Twig blight**

Mc Millan and Graves (1993) reported periwinkle twig blight caused by *Colletotrichum dematium* from Florida. The disease symptoms consisted of wilting of the shoot tips followed by chlorosis and eventual necrosis of the shoot tips. Necrotic tissues were typically covered with masses of acervuli with setae. The isolate produced falcate conidia as well as abundant sclerotia on the host and in culture.
Bacterial diseases

i. Shoot yellowing

Tang and Faan (1987) reported that Vinca rosea (Catharanthus roseus) was infected by bacteria like organism (BLO) producing distinct shoot yellowing symptoms. In cross transmission test, typical symptoms were produced on citrus plant as well as on C. roseus. The BLO existed in phloem cells of diseased plants and were measured 360-600 nm in length and 100-120 nm in width with a unit membrane of 25-30 nm thickness.

ii. Die-back

Ueno et al. (1998) reported from Brazil that Catharanthus roseus infected by Xylella fastidiosa produced small leaves, short internodes and die-back symptoms. Microscopic observation of infected plant sap revealed the presence of a large number of slender and rod shaped bacterial cells. The bacteria were isolated on buffered cysteine-yeast extract and periwinkle wilt agar media. Typical colonies of X. fastidiosa were observed 10 days after isolation on both the media. Immuno-fluorescence test with antibody specific to X. fastidiosa confirmed X. fastidiosa as the causal organism of the disease.

Phytoplasma diseases

i. Little leaf

Little leaf disease of Catharanthus roseus, which is characterised by virescence, reduction in the size of leaves and flowers, greening of flowers, shortening of internodes, extensive branching with numerous small leaves, stunting and failure of fructification is caused by a mycoplasma-like
organisms (MLO) localized in the phloem (Kar et al., 1982). Rao et al. (1983) also reported association of mycoplasma like bodies with little leaf disease of periwinkle in India. Pathogen could be transmitted in 20 days by grafting, inducing typical little leaf and phyllody in young C. roseus seedlings. Pleomorphic bodies, mainly circular and resembling mycoplasma were observed in phloem sieve cells of the diseased plant. Dafalla and Cousin (1988) reported the natural occurrence of virescence disease on Catharanthus roseus and Zinnia elegans from Sudan (Gezira). Virescence symptoms comprised both foliar and floral abnormalities. In both hosts, florescence and electron microscopic observation revealed the presence of mycoplasma like organisms (MLOs) in sieve tubes of diseased but not healthy plants.

ii. Witche's broom

Yang (1982) reported the transmission of Witche's broom disease from infected Ipomoea obscura to healthy Vinca rosea by means of Cuscuta chinensis. Infected V. rosea (C. roseus) developed severe yellowing, little leaf and extremely bushy Witche's broom symptoms, which were distinct from the mild symptoms induced by the sweet potato Witche's broom. Marwitz et al. (1987) reported mycoplasma like organisms (MLOs) from Witche's broom disease. Vaccinium myrtillus was transmitted for the first time to C. roseus using Cuscuta subinclusa as a vector causing yellowing of leaf blades and veins, a change of leaf shape from roundish-oval to oblong, diminishing leaf size, undulation of leaf blade margins, diminishing of the size of the flowers that did not open completely, shortening of flowers petals that become overlapped, no change in flower colour, and no flower phyllody. Comparison
of these symptoms with those of other MLO disease symptom in *C. roseus* indicated that witch's broom disease of *V. myrtillus* is different from all other plant MLO diseases known so far (Marwitz et al., 1987).

iii. Alder yellows

Alder yellows phytoplasma (ALY) was reported to be transmitted to *Catharanthus roseus* via dodder (*Cuscuta odorata*) bridges from naturally infected *Alnus glutinosa* trees. The identity of the dodder-transmitted phytoplasma was confirmed by restriction fragment length polymorphism (RFLP) analysis of PCR-amplified ribosomal DNA (Marcone et al., 1997).

Various diseases caused by *Rhizopus* species on different hosts

i. Damping off/Seedling rot

Gaur et al. (1989) observed a serious pre- and post-emergence seedling rot of groundnut in Sri Ganganagar in India. Isolations from infected seedling yielded *Rhizopus oryzae* and typical symptoms were reproduced on inoculated seedlings. Occurrence of seedling damping-off of rice plants caused by *Rhizopus* sp. was reported by Yaoita et al. (1984). Infected seedlings were found completely blighted at high temperature while hypertrophy or other abnormalities appeared in the proximal coleoptiles of infected seedlings at normal temperature.

ii. Fruit rot

Fruit rot disease caused by *Rhizopus* spp. has been reported on various hosts from different places in India and Nigeria. Ali and Shukla
(1981) reported a soft rot of brinjal fruit from Gwalior. The pathogen isolated from the necrotic lesion was confirmed to be *Rhizopus oryzae*. In nature, the disease starts as a soft watery rot accompanied by necrosis on the infected brinjal fruits, which become brown in advance stage of infection. Kumar *et al.* (1986) have described the symptoms of fruit rot of brinjal caused by *Rhizopus stolonifer* as yellowish brown water soaked lesions on the skin of the fruit which turned the whole fruit soft and pulpy within 2-3 days. The skin becomes loose into depressed area. The mycelium of the fungus with black sporangia covered the entire fruit surface.

Adisa and Fajola (1982) reported that soft rot of pineapple was caused by *Ceratocystis paradoxa*, *Rhizopus stolonifer and R. oryzae* in Nigeria causing 93% loss.

Singh and Chohan (1984) reported that *Aspergillus* sp., *Curvularia* sp., *Geotrichum* sp., *Myrothecium* sp. and *Rhizopus oryzae* were associated with fruit rot disease of cucurbits. *Rhizopus oryzae* produced water soaked lesions on fruit surface, which was covered with profuse growth of dirty whitish mycelium with scattered black pin head-sporangia. Isolations were made on potato-dextrose agar (PDA) and pure cultures were maintained on PDA slants. The pathogenicity of *R. oryzae* was established by inoculating injured as well as uninjured healthy fruits.

Adisa (1985) isolated *Rhizopus oryzae*, *R. stolonifer*, *Erwinia* sp., *Cladosporium* sp, *Fusarium oxysporum*, *F. equiseti*, *Aspergillus fumigatus*, *A. flavus* and *Penicillium multicolor* from *Capsicum annum* and *C. frutescens* fruit in the field in transit and in storage. They accounted for 40-45% and 25-35% of the losses in the wet and dry seasons, respectively.
Gupta and Pathak (1986) detected papaya fruit rot caused by *Aspergillus flavus*, *Rhizopus oryzae* and *Fusarium equiseti* in the markets of Jaipur. Later, Badyal (1991c) gave the details on the fruit rot of papaya caused by *Geotrichum candidum*, *R. oryzae* and *Fusarium moniliforme*.

Badyal (1990) has reported for the first time that *Rhizopus oryzae*, *Geotrichum candidum* and *Alternaria alternata* in Indian region spoiled fruits of *Docynia indica* (crab apple). Pathogenicity was confirmed by pinprick inoculation. During 1991, she investigated on post harvest fruit rots of sweet cherry *Prunus avium* L. Rotting of the fruits observed in the Jammu fruit market was caused by *Rhizopus oryzae*, *Paecilomyces variotii*, *Geotrichum candidum* and *Verticillium lateritium* (Badyal, 1991b). In another report (Badyal, 1991a), she gave the descriptions of the soft rot of almond and walnut caused by *Rhizopus oryzae* and *R. stolonifer*, respectively. Both green and dry almonds were affected by *Aspergillus ustus*. Walnuts were spoiled by a dry rot, caused by *Botryodiplodia theobromae* and *Penicillium italicum* (Badyal, 1991a). She reported the market disease of cucumber (*Cucumis sativus*) from Jammu. *Aspergillus niger*, *A. flavus*, *Rhizopus oryzae* and *Cladosporium oxysporum* were isolated from rotted cucumber fruits. Similar symptoms in pathogenicity tests were observed (Badyal, 1992). She reported a new fruit rot disease of tomato due to infestation of *Penicillium expansum*, *R. oryzae*, *Trichoderma koningii* and *Pestalotiopsis* sp. Pathogenicity was confirmed by inoculations using the prick stick method (Badyal, 1994).

During 1990-1992, Badyal and Sambali (1990a,b,c; 1992) surveyed the different areas of India. They found that different fruits, such as peach, mango, sapodilla and guava, were infected with fruit rot disease caused by *Botryodiplodia theobromae*, *Rhizopus oryzae*, *Penicillium crustosum*, *P.
italicum, P. chrysogenum, Geotrichum candidum, Cladosporium oxysporum and Ceratocystis adipose. The typical symptoms were water soaked lesions, which gradually increased in size and covered the whole fruit. Under the humid conditions, white fluffy growth with black sporangial heads enveloped the entire fruit making it loose in shape and texture.

Ekundayo and Oso (1993) reported the fungal spoilage of fresh pepper (Capsicum annum L.). Fresh pepper fruits were artificially inoculated with Aspergillus flavus, Botryodiplodia theobromae and Rhizopus oryzae to investigate the mode of entry of these pathogens into the plant tissues. The fungi gained entrance through wounds and points of attachment of the stalk to the fruit. The rot occurred at 10-40°C, while at higher temperature, the fruit started dehydrating, which affected the infection process. Protection of pepper fruits against injuries during harvest and cold storage was recommended to prevent post harvest decay.

Ray and Mishra (1995) noticed the spoilage of sweet potato tubers in Orissa and analysed the presence of microorganism associated with spoilage. Pathogens isolated included Erwinia sp, Botryodiplodia theobromae, Cochliobolus lunatus, Rhizopus oryzae, Fusarium oxysporum and F. pallidoroseum. However, other fungi were also identified, including Aspergillus terreus, A. flavus and A. fumigatus, but thought to be secondary invaders.

iii. Head rot

Head rot disease of sunflower caused by Rhizopus oryzae has been reported from different parts of India, Australia, Italy, USA and Israel. Middleton (1977) reported from Queensland (Australia) that the head rot of
sunflower was caused by *R. oryzae*. Disease occurred most frequently under high humidity. Roger *et al.* (1978) from Texas (USA) established a correlation between head rot disease and infestation by larvae of the sunflower moth (*Homoeosoma electellum*). Zazzerini and Tosi (1984) reported the association of two new parasites, namely *R. oryzae* and *Orobanche ramosa* with sunflower head rot disease from Italy.

Gulya *et al.* (1991) reported from USA that sunflower seed production has shifted from the north central states of North Dakota and Minnesota to California, primarily because of the longer growing season, but also to escape disease and insect problem of the Midwest. The most prevalent diseases observed between 1983 to 1988 were head rot (*Rhizopus oryzae*), powdery mildew (*Erysiphe cichoracearum*), and charcoal rot (*Macrophomina phaseolina*), rust (*Puccinia helianthi*) and wilt (*Sclerotinia sclerotiorum*). Survey during 1989 detected *Rhizopus* head rot in 71%, rust in 30%, powdery mildew in 25%, wilt in 20% and charcoal rot in 8% of surveyed fields.

Kushal and Saharan (1994) reported from Haryana (India) that *Rhizopus oryzae, Sclerotinia sclerotiorum* and *Aspergillus parasiticus* caused 68-78% inhibition in seed germination. Kushal *et al.* (1995) again reported from Haryana that severely infected seeds of sunflower reduced seed viability by 61.6%.

Kumar *et al.* (1996) observed that the seed health impairment was caused by *Rhizopus* head rot in sunflower. Disease was severe during spring season and *R. oryzae* had an 84.2% frequency of occurrence whereas other fungi associated with the rot had very low frequency. The fungus was recovered from the outer pericarp; inner pericarp, endosperm and embryo of
infected seeds and was capable of causing severe seed rot. It caused 8.5 to 11.5% seedling mortality. Infected seeds showed a significant reduction in oil content, which deteriorates the quantity of seed as well as economic loss in terms of quantity of the oil.

Shtienberg (1997) reported from Israel that when heads of sunflower were inoculated at the budding stage, loss was not apparent, because inoculated heads were not infected. When inoculation was done at the anthesis stage, loss was relatively high (42.5 to 99.1%) and when heads were inoculated at the seed development stage, yield was not reduced significantly.

**iv. Seed rot**

Pelcz et al. (1983) observed that seed infection by *Rhizopus oryzae*, reduced germination and seedling emergence in onion (*Allium sepa*). No differences in infection were found between onion cultivars and wild *Allium* spp.

Wahid et al. (1994) reported from Pakistan that the different fungi were associated with seeds of different cotton varieties. Five pathogens viz., *Rhizopus stolonifer*, *Fusarium moniliforme*, *F. orysporum*, *F. solani* and *Cercospora gossipina* were observed in high frequencies and their maximum infection was 100.0, 28.0, 17.0, 3.0 and 6.5 %, respectively on fuzzy seeds.

**v. Stem rot**

Wilson et al. (1983) noted that *Rhizopus arrhizus* incited stem rot of *Nicotiana glauca*. In the field, infected plants produced a slimy wet rot of the cortical tissues that become pale to yellowish brown when dry. The disease
often caused the flower heads to bend downward. The fungus also infected several detached fruits and vegetables including cotton (*Gossypium hirsutum*) bolls and sunflower (*Helianthus annuus*) heads when artificially inoculated.

Moura (1987) from Brazil reported wet rot, a new disease of yam (*Dioscorea cayennensis*) caused by the *Rhizopus oryzae*. Symptoms were an initial wet necrosis on the cortex, almost always in the growth zone of the tuber, followed by the break down of the internal tissue with a white mycelium appearing on the necrotic area, particularly during storage. Pathogenicity of *R. oryzae* was confirmed by inoculation. The disease was found on tuber from soil with high moisture content during periods of heavy rainfall.

**Taxonomical studies in the genus *Rhizopus***

The taxonomy of the genus *Rhizopus* Ehrenberg is controversial, and general accepted scheme for species delineation has not yet emerged. Inui *et al.* (1965) divided the genus into sections on the basis of temperature responses. The section 'Oryzae' consisted of species growing well at 37°C but not at 45°C in Pfeffers’ solution. Dabinett and Wellman (1973) applied numerical methods on data published by Inui *et al.* (1965) and arrived at an essentially similar classification.

Scanning electron microscopy (SEM) was used to analyse finer surface details and several pattern types based on sporangiospore shape and surface striation were described (Ellis *et al.*, 1970). Surface striations were later used in many studies (Domsch *et al.*, 1980). With more satisfactory specimen preparation techniques, Ellis (1981) extended the earlier SEM observations
and concluded that sporangiospores could be an important character for species identification and classification. Seviour et al. (1983) utilised SEM and cultural physiology on *Rhizopus* strains' data to assess their taxonomic applicability in delimiting species of the genus *Rhizopus*. SEM of the sporangiospores of a variety of isolates indicated that there was a continuum of patterns ranging from almost featureless surfaces to verruculose and deeply ridged or striated topologies. Cultural characteristics, radial growth rate, sporulation pattern, growth and viability at raised temperatures were found too variable for use in taxonomic studies.

Zygospore formation was once thought to be a useful criterion for taxonomic purposes (Scholer et al., 1983), but since intraspecific incompatibility or even the complete absence of a sexual stage occurs in many species, emphasis were given to asexual and (or) physiological and cultural characters for classification purposes. Of these, sporangial, sporangiophore, sporangiospore shape, dimension and orientation were considered important characters, as was the maximum temperature for growth (Scholer et al., 1983).

**General characteristic features**

*Rhizopus Ehrenberg (1820)*

Sporangiophores mostly formed on stolons opposite to rhizoids, either single or more, often in clusters, unbranched, occasionally divided near the top, bearing multispored, terminal sporangia. Sporangia globose distinctly columellate, apophysate, greyish to brownish at maturity. Sporangiospores (sub) globose to ellipsoidal and angular. Zygospores covered with spines or
warts, formed in aerial mycelium between non-ornamented, isogamous, opposite suspensors (Vuillemin, 1902; Lind, 1913).

*Rhizopus* differs from *Mucor* Mich.: Fr. and *Phycomyces* Kunze.:Fr. by having stolons and rhizoids. It can be separated from *Actinomucor* Shostakovich by dark coloured sporangia on unbranched sporangiophores, mostly arising from well-developed stolons with distinct rhizoids (Schipper, 1984).

i. *Rhizopus stolonifer*

Asexual structure

Common features of the majority of the strains of the *R. stolonifer* group are as follows: Rhizoids are well developed and sporangiophores are mostly up to about 2000 µm in length and 20-25 µm in diameter. Sporangiospores are angular-subglobose to ellipsoidal, with distinct ridges on the surface. Sporangia are up to about 250 µm in diameter; length of columellae slightly more than half of the sporangial height and the larger columellae are conical-cylindrical in shape with mouse grey or brownish colour. No growth is observed at 33°C (Schipper, 1984).

The homothallic species *Rhizopus sexualis* resembles *R. stolonifer* in the shape and size of its sporangiospores, but differs in having relatively small sporangia (including columellae) and small sporangiosphores. *R. sexualis* var. *americanus* has somewhat different sporangial state, but should be regarded as a close relative because of similar zygosporic stages and temperature responses (Schipper, 1984).
Zygospores

The species of the *Rhizopus stolonifer* group are both heterothallic and homothallic and produce black, relatively large zygospores. The ornamentation of the outer spore wall differs widely from that of the *R. microsporus* and *R. oryzae* groups. Within the *R. stolonifer* group morphological differences in the zygosporic stages are found in the size and shape of the suspensor pairs (Schipper, 1984). During his study, comparatively small and seemingly “unfinished” zygospores were often found, well-developed ones were comparatively rare. Even the large sized black zygospores were irregular with frayed projections and slightly angular bodies.

Namyslowski (1906) described a homothallic strain of *R. stolonifer* producing zygosporic colonies from single vegetative spore isolations, if grown under favourable conditions. Many parthenospores and incomplete conjugation were also observed. *Rhizopus sexualis* is a homothallic species resembling *R. stolonifer* in several respects. Callen (1940) contrasted the type strain of *R. sexualis* with (+) and (-) strains of *R. stolonifer* and obtained “hybrid zygospores” in both cases, though most abundantly with *R. stolonifer* (+). He did not regard them as true hybrids but only as mixochimeres with an association of partnership of nuclei. Germination could not be obtained.

Schipper (1978) considered Callen’s “hybrid-zygospores” as induced parthenospores. Induced parthenospores develop from one gametangium only, as lysis of the fusion-wall between gametangia does not occur; consequently these products need not indicate relationships or specific identities of the strains involved. Hawker and Syrop (1973) established
that zygospore initiation and development in *R. sexualis* were poor at temperature below 10°C. Zygospore production is favoured at 90% relative humidity or above (Harris and Dennis 1980).

## ii. *Rhizopus oryzae*

### Asexual structure

Common features of most strains of *Rhizopus oryzae* were: rhizoids of medium size with sporangiophores up to 1000-1500 µm in length, (10-) 13-15 (-20) µm in width and local swellings and dichotomous branching were present. Sporangia were up to 150-175 µm in diameter and columellae were ellipsoidal on a truncate base and mouse-grey or brownish in colour. Sproangiospores were angular, subglobose to ellipsoidal, with ridges on the surface, up to 8 (-10) µm in length (Schipper, 1984).

### Zygospores

In *Rhizopus oryzae* the zygospores were reddish brown when young, then brown, stellate with conical projections, up to 140 µm in diameter between unequal suspensors (Schipper, 1984).

Table 2 summarises the differences between *Stolonifer* and *Oryzae* group.
Table-2: Diagnostic characters of *stolonifer* and *oryzae* group  
(Schipper, 1984)

<table>
<thead>
<tr>
<th>Characters</th>
<th>Stolonifer group</th>
<th>Oryzae group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizoids</td>
<td>Complex, well developed</td>
<td>Medium</td>
</tr>
<tr>
<td>Sporangiospores</td>
<td>1-3 (4) mm long</td>
<td>Maximum 1-2.5 mm long</td>
</tr>
<tr>
<td>Sporangia</td>
<td>(150) 250-275 (300) μm in diameter</td>
<td>Maximum diameter 160-240 μm</td>
</tr>
<tr>
<td>Zygospores</td>
<td>Black, upto 225 μm in diameter</td>
<td>Brown, upto 140 μm in diameter</td>
</tr>
<tr>
<td>Suspensor</td>
<td>Equal (approx.)</td>
<td>Unequal</td>
</tr>
<tr>
<td>Maximum growth</td>
<td>(33) 36°C</td>
<td>45°C</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occurrence biology</td>
<td>On overripe fruit</td>
<td>Food fermentors; agents of mucormycosis</td>
</tr>
</tbody>
</table>

**Factors affecting the growth and sporulation of *Rhizopus oryzae***

It is commonly known that most of the diseases appear and develop best during wet warm days, especially after rains. Plants heavily fertilized with nitrogen are usually much more severely attacked by the pathogen than less fertilized plants (Bawden and Kassanis, 1950). This clearly indicates that environmental conditions prevailing both in the air and soil, affect greatly the development of the disease and also determine whether a disease will occur or not. The environmental factors that most seriously affect the initiation and development of typical symptoms of the diseases are; temperature, moisture, light, soil nutrients and soil pH. A combination of
four factors viz., susceptible plants, virulent pathogen, favourable environment and appropriate time are essential for a disease to occur and to develop optimally (Dwivedi et al., 1992). However, although plant susceptibility and pathogen virulence remain essentially unchanged in the same plant for at least several days, and some times for weeks or months, the environmental conditions may change more or less suddenly and in various degrees. Such changes influence the development of the disease in progress, or the initiation of new ones, more or less drastically. Plant diseases generally occur over a fairly wide range of the various environmental conditions. Nevertheless, the extent and frequency of their occurrence as well as the severity of the disease on individual plants are influenced by the degree of deviation of each environmental condition from the point at which disease development is optimal (Miller, 1953).

Thakur (1973) emphasized on the factors influencing storage rot of certain fruits, vegetables caused by species of Rhizopus. Infection developed rapidly at higher temperatures (20-40°C) and 50% and above relative humidity (RH).

Dennis and Hocker (1981) worked on the effect of relative humidity on chilling sensitivity of sporangiospores of Rhizopus species. Sporangiospores of R. stolonifer, R. arrhizus and R. oryzae maintained viability at 75-100% RH. At 66% RH, spores of R. stolonifer lost viability at either 0°C or 20°C and those of the other two species at 20°C only. The viability of R. sexualis was affected by temperature over the whole RH ranges and a marked loss in viability occurred at 100% RH at 0°C but not at 20°C. The sporangiospores were more sensitive to chilling when exposed to low temperatures in aqueous solutions.
Akushie and Clerk (1981) determined the effect of relative humidity on viability of *Rhizopus oryzae* sporangiospores. Longevity at 20°C, 25°C and 30°C was approximately the same at each relative humidity (RH). The most favourable RHs for survival were 0% and 100%. After 40 days in storage, while > 65% of sporangiospores were still alive at these RHs, there was practically no viable spore at 20-80% RH. The two most destructive points 0-40% and 80% were observed in contrast with results obtained with other fungi, which had a single destructive point only.

Wilson *et al.* (1983) noted that maximum linear growth of the *Rhizopus arrhizus* on potato-dextrose-agar and reported maximum decay in cucumber (*Cucumis sativus*) to occur at 35°C.

The effect of some environmental factors on the growth and pathogenicity of six pineapple fruit rot pathogens has been reported by Adisa (1983). Among the six pathogens, *Rhizopus oryzae* and *R. stolonifer* did not grow at 0°C, but there was a gradual increase in growth at 10-30°C. Optimum pH range was observed from 6.0 to 8.0. There was no spore germination at 0% RH.

Abdel Rahim *et al.* (1983) reported that in sugarcane plants, fungal activities increased with plant age. Fungi isolated from both rhizosphere and non-rhizosphere soils were predominantly belonged to *Aspergillus* and *Rhizopus* species.

Kaul (1986) emphasized on the production of mass culture and sexual fructification of *Rhizopus stolonifer* in modified Richard's liquid medium. He concluded that Richard's medium with ammonium sulphate was the only liquid synthetic medium that could support maximum mycelial growth and induce fructification resulting in mass culture production of *R. stolonifer*. 
Rhizopus oryzae, a mucoraceous fungus caused soft rot of potato in storage. The optimum temperatures for growth of the fungus in culture and on tubers were around 35°C, while the favourable relative humidity was 75-85% (Ray et al., 1997).

Odebode and Unachukwu (1997) mentioned the effect of storage environment on carrot root rots and biochemical changes during storage. The optimum temperature and relative humidity for the development of carrot root rot during storage were found to be 20°C and 60%, respectively and rot development increased as these environmental factors increased. Both temperature and relative humidity enhanced the biodeterioration of the carrot roots through an increase in the diameter of the rotted area of infected tissue. The storage rot was caused by Rhizopus oryzae, Trichoderma harzianum, Botryodiplodia theobromae, Aspergillus niger and A. flavus. The level of total soluble sugar in rotted carrot root was substantially decreased in 2-4 days after infection. Paper chromatography showed the presence of glucose, maltose, sucrose, lactose and galactose in healthy carrot roots, while only lactose and galactose were present in infected roots. The ascorbic acid, total nitrogen, crude protein, crude fibre, fat and mineral contents in infected carrot roots reduced as the storage period increased.

Production of toxins and their role in plant diseases

Living plant cells are complex systems in which many interdependent biochemical reactions take place concurrently or in a well-defined succession resulting in the intricate and well-organized processes essential for life. Disturbance in any of these metabolic reactions causes disruption or shift of the physiological processes that sustain the plant and leads to development.
of disease. Among the factors inducing such disturbances are toxins — the metabolites that are produced by plant pathogenic microorganisms and act directly on living host protoplasts, seriously damaging or killing the cells of the plant (Agrios, 1997). In plant pathology, a toxin is generally defined as a non-enzymatic substance that injures plant cells or disrupts their metabolism (Owens, 1969). Toxins are effective in very low concentrations and are of lower molecular weight than animal toxins, and in most cases do not induce antibody formation when injected into animals (Wood, 1976).

The available information indicates that toxins injure the host cells either by affecting osmotic relationships of the cells or by affecting enzymatic reactions going on in the cytoplasm. A change in osmotic relationships can be brought about by alteration of the ionic balance of a cell by the action of toxin on the structure of the cell membrane resulting in decrease or increase in membrane permeability. The enzymatic reactions in plant cells can be affected either through the chelating properties of some toxins, through reaction between the toxin and the enzyme, and subsequent interruption of the corresponding enzymatic reaction or through excessive accumulation of normal metabolites. Certain toxins are known to act as anti-metabolites inducing a deficiency for an essential growth factor. Thus, most toxins seem to have a direct or indirect effect on the respiration of the plant, which again may increase or decrease, depending on the toxin and on the particular step of respiration affected (Owens, 1969).

Although phytotoxins act primarily on plants, their effect may extend beyond the plant kingdom, for example to certain microorganisms or animals. A number of phytotoxins have been reported to be antibiotics or mycotoxins that exhibit antimicrobial activity or, if ingested with
contaminated food, may be hazardous for animals and humans. Since phytotoxins are biologically active compounds, a variety of applications have been successfully attempted. Currently, phytotoxins are being used as; probes for the rapid screening of plant clones or the progeny from crosses for resistance to disease, promising agents for the biological control of noxious organisms, promoting the germination of dormant seed or to speed up the drying of leaves or hay. As the knowledge about the occurrence and range of sensitive organism expands more utilization of phytotoxins as biocides may become possible. (Graniti, 1991).

Toxins may or may not follow the same host specificity. A toxin is termed host specific when it has high biological activity towards that plant only, which is also host to the pathogen that produces it, conversely, a toxin is termed non-host-specific when it affects a wider range of plant species. The biological role of a toxin is the main criterion for its further classification either as a pathogenicity factor i.e., a toxin that is essential for a pathogen to cause disease or as a virulence factor i.e., a toxin that increases the extent of disease. Toxins that are considered virulence factors generally turn out to be the non-host-specific toxins, whereas pathogenicity factors generally are host-specific toxins (Scheffer and Briggs, 1981).

**Host specific toxins**

i. **HV toxin**

Meehan and Murphy (1947) first reported the production of a toxin in culture filtrate of the *Helminthosporium victoriae*, which caused victoria blight on oat. The toxin was found to be host-specific and only susceptible
seedlings were killed while resistant varieties remained unaffected. This toxin was later known as one of the best example of host specific vivotoxin.

ii. **HC toxin**

*Helminthosporium carbonum* is closely related to *H. victoriae* that is pathogenic to certain maize hybrids. This pathogen has also been found to produce a host-specific toxin which is being referred to *H. carbonum* toxin (HC toxin) (Pringle and Scheffer, 1967).

ii. **Helminthosporium maydis toxin**

*Helminthosporium maydis* was reported to cause devastating destructive disease in Texas male sterile corn. Symptoms induced by the fungus, included water soaked lesions and chlorosis. *H. maydis* produced a host-specific toxin that was isolated and tested on the corn seedlings. The symptoms produced by treatment of host plant with toxin and the pathogen were identical and expressed the same specificity. Corn hybrids having Texas male sterile cytoplasm was susceptible, while varieties, which did not contain male sterile cytoplasm, were resistant to the toxin and the pathogen (Smedgard-Peterson and Nelson, 1969).

iv. **Helminthosporal toxin**

*Helminthosporium sativum* (*Cochliobolus sativus*) caused seedling blight, root-rot and leaf blight diseases on wheat and barley. Ludwig (1960) showed that the fungus produced chlorosis and necrosis on the seedlings of the host plants and the disease symptoms were attributed to a toxic metabolite produced by the pathogen. This toxin was later isolated and
characterised by De Mayo et al. (1961) and was identified as a sesquiterpenoid helminthosporal.

**Non-host specific toxins**

**A. Bacterial phytotoxin**

**i. Tabtoxin**

"Wildfire" is a highly infectious leaf spot disease of tobacco caused by *Pseudomonas tabacii*. The characteristic symptoms of the disease consist of necrotic spots surrounded by chlorotic halos and chlorosis of the leaves. Cell free culture filtrate when applied on healthy leaves produced symptoms identical to natural infection, suggesting the possibility of the production of a toxic metabolite, which may be responsible for the chlorosis (Braun, 1955). Woolley et al. (1952) first isolated this toxin and proposed a structure that was later withdrawn and Stewart (1971) published an acceptable structure. Tayler et al. (1972) proposed a name "tabtoxin" to the wildfire toxin.

**ii. Phaseotoxin**

*Pseudomonas phaseolicola* that also causes chlorotic halo in the infected bean plants is known to produce a phytotoxin, phaseotoxin. The halo inducing phytotoxin was isolated by Rudolph and Stahmann (1966) from *in vitro* and *in vivo*, and was partially purified. The phytotoxin induced chlorosis in the host and caused an accumulation of ornithine and also inhibited the growth of beet leaves. The phytotoxin was reported to be thermostable and non-specific in nature.
iii. Rhizobiotoxin

Some strains of the legume root bacterium *Rhizobium japonicum* were reported to fix nitrogen in the normal manner and simultaneously synthesise a toxin that induced chlorosis on the leaves of host plant soybean. The toxin was isolated from culture as well as from diseased plants (Owens and Wright, 1965).

B. Fungal phytotoxin

i. Colletotin

*Colletotrichum fuscum* caused necrosis and water soaked spots on *Digitalis lanata* and *D. Purpurea*. The disease symptoms suggested the possibility of role of a toxic metabolite that was later isolated and characterised as the toxin colletotin, and was identified as glycopeptide containing a peptide and polysaccharide (Goodman, 1960).

ii. Fusicoccin

*Fusicoccum amygdali*, pathogenic to almond and peach caused wilting and dessication of infected plants. The fungus produced a toxic metabolite that was reported to induce symptoms on peach cuttings similar to natural infection (Graniti, 1962). Ballio *et al.* (1964) isolated and characterised a phytotoxic diterpene glucoside with a molecular weight of 680 from the fungus *F. amygdali* and named it as fusicoccin. Fusicoccin induced rapid wilting of leaves followed by dehydration. The toxin stimulated water uptake due to increased transpiration, cell wall enlargements and etiolated pea internode segments.
iii. **Phytophthora toxins**

Phytophthora parasitica produced a toxic metabolite that caused necrotic spot on the leaves and was thought to be a polysaccharide (Ballio and Gianani, 1972). *P. megasperma* var. *sojae* causing root and stem rot of soybean also produced a toxin, which induced wilting of seedlings of the host (Paxton, 1972).

iv. **Alternaria toxin**

A metabolite, tenuazonic acid isolated from *Alternaria alternata* (Fr.) Keissler causing leaf blight of *Datura innoxia* Mill. showed significant phytotoxic activity when tested on monocot and dicot plants. The toxin produced chlorosis and necrosis on leaves of *D. innoxia*, *D. stramonium*, *D. metel*, belladonna, cowpea, wheat, rye, cabbage, cauliflower and maize at 200 μg/ml and wilting on seedlings of *D. innoxia* at 100 μg/ml concentration. It was a non-specific phytotoxin and appeared to play significant role in pathogenesis (Janardhanan and Husain, 1984). Several other toxins are produced by other *Alternaria* species. (Pero and Main, 1970; Janardhanan and Husain, 1974; Tietjen et al., 1983).

v. **CA toxin**

Alam et al. (1997) reported that *Curvularia andropogonis*, the causal agent of leaf blight of Java citronella, produced a phytotoxic metabolite in culture (*in vitro*) as well as in plants (*in vivo*). Phytotoxin was isolated from 13-day-old culture filtrate of the fungus and from infected leaves of the host plant 7-10 days after inoculation with a pathogenic strain (87 ca-1) of *C. andropogonis*. A batch of 5 litres inoculated glucose-salt medium and 500 g
infected leaf tissue with necrotic lesion yielded 4.27 g and 200 mg crude phytotoxin, respectively. The phytotoxins were purified from in vitro and in vivo through gel filtration on sephadex G-10 and thin layer chromatography (TLC) on silica gel G. Purified phytotoxin induced symptoms of the disease on the host plants even at a low concentration (500 μg/ml). Phytotoxin was reported non-host specific, heat stable and hygroscopic in nature. Phytotoxin was characterised by UV, IR, MS and NMR spectral analysis and identified as 1-0-β-D (14-hydroxy-4, 12-eicosadienoyl)-glucoside.

vi. Rhizopus toxin

Very little work has been done on toxin produced by Rhizopus species Mirocha et al. (1961) isolated fumaric acid from fruits of almond infected by Rhizopus spp. and examined its role in the involvement of disease syndrome. The fungus was restricted to the rotting fruit but the shoot bearing the fruits were completely blighted. It was indicated that toxin produced by Rhizopus spp. moved from the site of infection to the shoot resulting in premature death and drying of shoots.

Production of pectic enzymes and their role in plant diseases

During plant infection many parasitic microorganisms produce extracellular cell wall degrading enzymes (Cooper, 1984). However, a role in pathogenesis has been established only for pectic enzymes. These enzymes degrade the pectic component of the middle lamella and of the primary cell wall, facilitating pathogen penetration and colonization (Collmer and Keen, 1986; Bateman and Miller, 1966). Pectin is the major component of the
primary plant cell wall and middle lamellae. It was reported that depolymerization of pectin was a prerequisite for further cell wall breakdown by other cell wall degrading enzymes (Karr and Albersheim, 1970).

**Endopolygalacturonase: production and characterisation**

Endopolygalacturonase is one among the well studied pectic enzymes, and it was found to be the first enzyme secreted by certain fungal pathogens grown on isolated plant cell walls (Jone et al., 1972). The importance of polygalacturonase (PG) in pathogenesis has been well established in some plant diseases (Bateman and Beer, 1965; Lei et al., 1985; Rodriguez-Palenzuela et al., 1991), whereas in another report, no effects of PG on pathogenicity were found (Scott-Craig et al., 1990).

Production of polygalacturonase by *Endothia parasitica* was reported in culture and in blight cankers. It was suggested that oxalic acid, which was produced by the pathogen, served to chelate the calcium in polypectate exposing it to digestion by polygalacturonase (McCarroll and Thor, 1978 and 1985).

In the phosphate stimulated infection of french bean leaves by *Botrytis cinerea*, two polygalacturonases (PGs) namely PG1 and PG2, were reported to be accumulated 6-12 h after inoculation, in inoculum drops during fungal penetration of the outer epidermal cell wall (Van den Heuvel and Waterreus, 1985). Later reports on regulation of the synthesis of pectic enzymes of *B. cinerea* showed the sequential nature of their production (Leone and Van den Heuvel, 1987). The constitutively produced polygalacturonase isoenzyme (PG2) was isolated from culture filtrates of *B. cinerea*, purified to homogeneity and characterised (Leone et al., 1990).
PG2 hydrolysed sodium polygalacturonate more quickly than pectin. The optimal pH for PG2 activity with sodium polygalacturonate was 4.5 and with pectin, it was 4.0. PG2 activity was also influenced by the presence of NaCl or CaCl_2 in the reaction mixture. Analysis of the break down products by paper chromatography and a comparison of the reaction rate by viscometry and reducing group assay revealed that PG2 had an endocatatylyic mode of action on polygalacturonate. The isoelectric point and the molecular mass of PG2 were estimated to be 9.1 and 23.0 KD, respectively.

A single enzyme capable of degrading polygalacturonic acid has been isolated and purified from the culture filtrates of *Cochliobolus carbonum*. Production of the enzyme occurred only when the fungus was grown on pectin, but was stimulated three fold by the addition of 2.0 gl^-1 sucrose (Walton and Cervone, 1990). The enzyme acted in an endo fashion and preferred polygalacturonic acid to pectin as substrate. The enzyme was a glycoprotein in nature and had amino acid composition similar to other fungal polygalacturonases.

Gao and Shain (1994) carried out Endopolygalaturonase production and characterisation studies in the chestnut blight fungus. Extracellular endopolygalacturonase was produced by *Cryphonectria parasitica* in culture media supplied with 1% sodium polypectate. Production of the enzyme was stimulated dramatically by 1% glucose, 0.25% yeast-extract and 0.75% malt extract. The enzyme purification yielded apparently homogenous enzyme solution (244 fold purification). Molecular weight and isoelectric point of the enzyme was reported 42 kD as estimated by SDS-PAGE and gel filtration and 8.0, respectively. The pH optima for the enzyme activity were 4.5-5.0, and its optimum temperature was 40°C. The Michaelis constant (K_m) and
maximum velocity \(V_{\text{max}}\) of the enzyme were 0.22 mg ml\(^{-1}\) with polygalacturonic acid as substrate, and 0.241 milli-moles of reducing group min\(^{-1}\) mg\(^{-1}\) protein, respectively. The enzyme acted in an endo fashion; a 50% reduction in viscosity of polygalacturonic acid resulted in hydrolysis of fewer than 2.5% of the glycosidic bonds.

Gupta et al. (1997) studied the production of polygalacturanase (PG) from *Penicillium* sp. and its UV induced mutant. Mutant produced twice of the enzyme activity compared to the parent one. Maximum production of enzyme was observed after 120 h of incubation at 30°C in both. Among various agricultural by-products used as substrates (apple pomace, citrus peel, sugar beed and raw onion), highest activity was observed in sugar beet shred. At temperature optima of 45°C, the enzyme showed an optimum pH of 5.5 and was thermostable below 30°C upto 24 h. \(K_m\) of the enzyme for polygalacturonic acid was 7.69 mg ml\(^{-1}\). Hg\(^{2+}\) was found most potent inhibitor of enzyme that caused 100% inhibition at 1 mM concentration. Other inhibitors viz., EDTA, PMSF, Mg\(^{2+}\), Cu\(^{2+}\) and sodium azide caused inhibition ranging from 2% to 21%, while Mn\(^{2+}\) was reported stimulator that resulted in an increase in enzyme activity by 24%.

**Polygalacturonase production by Rhizopus species**

Cappellini (1966) studied the polygalacturonase production by *Rhizopus stolonifer* and its correlation with fungal growth. Enzyme activity was found, after an apparent lag, to increase sharply during active (logarithmic) growth and then, to diminish as the organism entered a decelerated growth phase. During logarithmic growth, total enzyme activity of the intra- and extra-cellular fractions increased 8-fold and 40-fold,
respectively, while the specific activities increased 5-fold and 20-fold, respectively. During initiation of decelerated growth phase, specific activities of both fractions dropped abruptly. Apparently, synthesis and active secretion of polygalacturonase occurred during the active growth phase and ceased prior to the attainment of maximum growth. The pectolytic enzyme system was found to be unstable in culture filtrate during growth and under certain storage conditions. The intracellular and extracellular pectolytic systems appeared similar that degraded sodium polypectate randomly to monogalacturonic acid.

The production and activity of polygalacturonase (PG) was assessed in apricot fruit at three ripeness levels by Luh et al. (1974). The effect of PG from *Rhizopus stolonifer*, *R. arrhizus* and *R. oryzae* was demonstrated in destroying the texture of canned apricots.

**Disease control**

Disease control is important for the sustainable economic production of most of the crops. Different ways to control plant diseases viz., regulatory, cultural, physical, chemical and biological depending upon the nature of the agents employed have been described (Agrios, 1997). Present review is emphasised on the chemical and biological control related studies.

**Chemical control**

Among the isolates of 17 genera, the most frequently recorded were *Rhizopus oryzae*, *Alternaria tenuis*, *Fusarium* sp., *Aspergillus* sp., etc. associated with sugar beet seeds. The fungal flora could be reduced
considerably by polishing the seeds, and eliminated by seed dressing with Arelan or Ceresan (1 g/kg seed) (Singh et al., 1974).

Rath and Mohanty (1978) worked on control of Rhizopus rot of storage garlic. Losses from R. oryzae were minimized by treating garlic bulbs with 0.1% mercuric chloride (if stored for seed) or 0.03% formalin (if stored for table use) at intervals of 10-15 days.

Mittal and Sharma (1982) studied the mycoflora and its control on the seeds of some forest trees, such as Shorea robusta, Pinus wallichiana, Pinus roxburghii. In Shorea robusta the most common fungi were Aspergillus niger, Penicillium canadense and Rhizopus oryzae and these were also responsible for seed deterioration. The most effective of 9 fungicides tested were; Agrosan GN, Brassicol, Captan, Dithane M-45 and Panoctine. In Pinus wallichiana, fungi causing deep-seated infection were Aspergillus funiculosus, A. niger, Fusarium semitectum and R. oryzae. Thiram and Dithane M-45 gave the best control of seed borne fungi. In Pinus roxburghii, fungi isolated from seeds included, Aspergillus niger, A. candidus, A. fumigatus, Fusarium oxysporum, Penicillium brevicompactum, P. canadense and R. oryzae. Thiram and Ceresan were reported significantly effective for their control.

Huang and Scott (1985) controlled the rotting and browning of litchi fruits by dipping the fruits in hot Benomyl (0.05-0.2% for 2-16 min) and packing loosely in sealed PVC bags. Aspergillus restrictus, A. niger, Rhizopus oryzae and R. arrhizus were present on rotted fruits. The recommended fungicide treatment for commercial use was 0.05% Benomyl at 52°C for 2 minute.

Singh and Singh (1989) worked out the efficacy of certain fungicides against Rhizopus oryzae causing rot of jackfruit. Bavistin, Benlate, Dithane
M-45, Dithane Z-78 and Elatox were tested for their effective control. Dithane M-45 proved highly effective in controlling the disease followed by Dithane Z-78 and Elatox. Bavistin and Benlate were found to be ineffective.

The production of amylase by *Rhizopus oryzae* from aubergine was completely prevented by Parasan at 0.025% and by Thiram, Fylotan, Brassicol, Captan and Dithane M-45 at 0.5%, 1.5%, 2%, 2% and 2%, respectively. Sulfex was ineffective even at 3% (Chaurasia, 1992).

Wahid *et al.* (1994) reported that out of twelve fungi recorded from 145 cotton seed samples collected from various districts of Punjab, five pathogens viz., *Rhizopus stolonifer, Fusarium moniliforme, F. oxysporum, F. solani* and *Cercospora gossypina* were in high frequencies. Samples with high infection of these fungi were delinted with 10% commercial sulphuric acid and given a pre-treatment with 0.1% Chlorax separately and in combination. All the treatments reduced the fungal count of the seeds.

Shtienberg (1997) suggested two approaches to be utilised for *Rhizopus* head rot management. One approach was to apply fungicides for suppression of the pathogen. However, in a set of experiments conducted by Shtienberg *et al.* (1996), copper-8-quinolinolate and six other fungicides were applied, but none reduced significantly the incidence of head rot. The other approach for management of *Rhizopus* head rot was to apply insecticides to control the insect that wound the heads. This approach has previously been evaluated on oil seed sunflower and on confectionery sunflower (Roger *et al.*, 1978; Klisiewicz, 1979; Shtienberg *et al.*, 1996).

**Biological control**

Ferrera Cerrato (1976) reported that *Trichoderma viride* parasitized several plant pathogens namely, *Alternaria solani, Rhizopus stolonifer, R.*
arrhizus, R. oryzae, Rhizoctonia solani, R. callae, R. endophytica and 
Macrophomina phaseoli (M. phascolina). Glucose and yeast extract at 10 g 
and 5 g/l strongly stimulated the parasitism.

Hunter et al. (1977) observed that Syncephalis californica frequently 
parasitized Rhizopus oryzae in nature on decaying apricot fruit on soil in an 
orchard. Parasitism occurred in vitro at 15-30°C, at pH values of 4.0-7.5 and 
at four different C: N ratios. Parasitism was observed in vitro and in non-
sterilised orchard soil at oxygen concentrations of 1-21% and at 
carbondioxide concentrations of 0.03-5%. Infection by S. californica 
suppressed sporulation of R. oryzae under moist conditions that were 
suitable for growth of the host. The result indicated that S. californica was an 
aggressive mycoparasite capable of attacking R. oryzae under a wide set of 
soil environments.

Dwivedi and Arora (1978) studied hyphal interaction among Rhizopus 
oryzae, a dominant soil inhabiting fungus and soil fungi with potential 
antagonistic characters, in vitro. Results depicted that diameter of the 
hyphae of interacting fungi played an important role in hyperparasitic 
interactions. None of the hyphae with wide diameter could penetrate inside 
and coil around the hyphae of R. oryzae, while most of the hyphae with 
narrow diameter did so.

Dwivedi and Mishra (1982) studied Rhizopus oryzae and 
Cladosporium cladosporioides isolated from the wheat field soil on agar 
plates for hyphal interaction. Blocks of agar (5 mm) of both the test fungi 
were placed aseptically 2 cm apart on 2 % Czapek-Dox-Agar (CZA) incubated 
at 25°C. Observations were made under a microscope by picking up the 
hyphae from intermingled colonies and staining them with cotton blue.
Hyperparasitic behaviour of \textit{C. cladosporioides} was observed on the hyphae of \textit{Rhizopus oryzae}. Coiling, penetration and ramification of hyphae of \textit{C. cladosporioides} were observed. The thickening of the host cell wall around the infection court and disappearance and granulation of cytoplasm were observed. Excessive growth in actively growing hyphae of \textit{C. cladosporioides} was perhaps due to the enormous nutrient available to them in the living part of the host of \textit{R. oryzae}. Ability of the host hyphae for the checking of growth of the parasite with plug formation provided an evidence for the defence mechanism during the hyperparasitism.

Endophytic bacteria were tested in the postharvest biocontrol of \textit{Monilinia laxa} and \textit{Rhizopus stolonifer} in stone fruit by Pratella et al. (1993). Of a total of 122 strains of these sub-epidermis microorganisms (endophytes) isolated from various hosts (cucumber, egg plant, pepper, tomato, zucchim, apricot, peach and plum), approximately twenty showed greater than 90\% control of the given pathogens. \textit{R. stolonifer} appeared to be less susceptible than \textit{M. laxa} to the endophytes.

Lima et al. (1997) selectively isolated many yeast and some yeast-like fungi from fruits and vegetables. In several assays performed on strawberries, table grape berries and kiwifruit, the yeast like fungus \textit{Aureobasidium pullulans} and the yeast \textit{Candida vanderwaltii} L60 and \textit{C. oleophila} L66 were the most effective antagonists of \textit{Botrytis cinerea} and \textit{Rhizopus stolonifer}. 

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